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Vol. 7. Part 1 April, 1961

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VETERINARY REVIEWS AND ANNOTATIONS

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COMMONWEALTH BUREAU OF ANIMAL HEALTH
WEYBRIDGE, SURREY
ENGLAND

Published by the
COMMONWEALTH AGRICULTURAL BUREAUX
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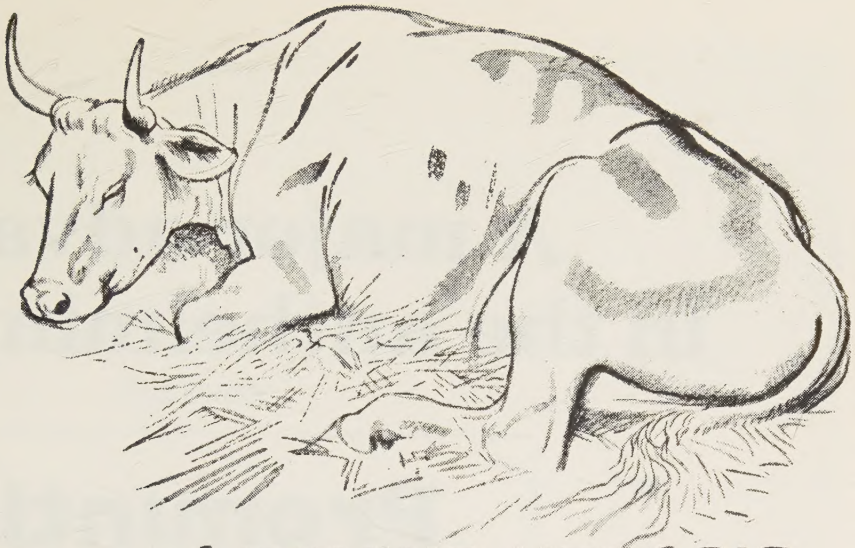

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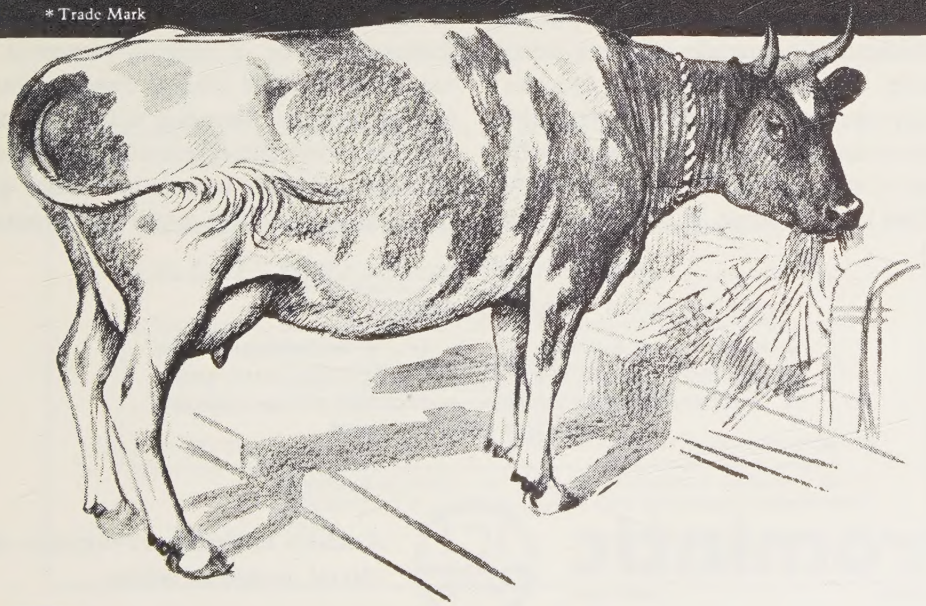

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CORRIGENDA — Vol. 6, Part 2.

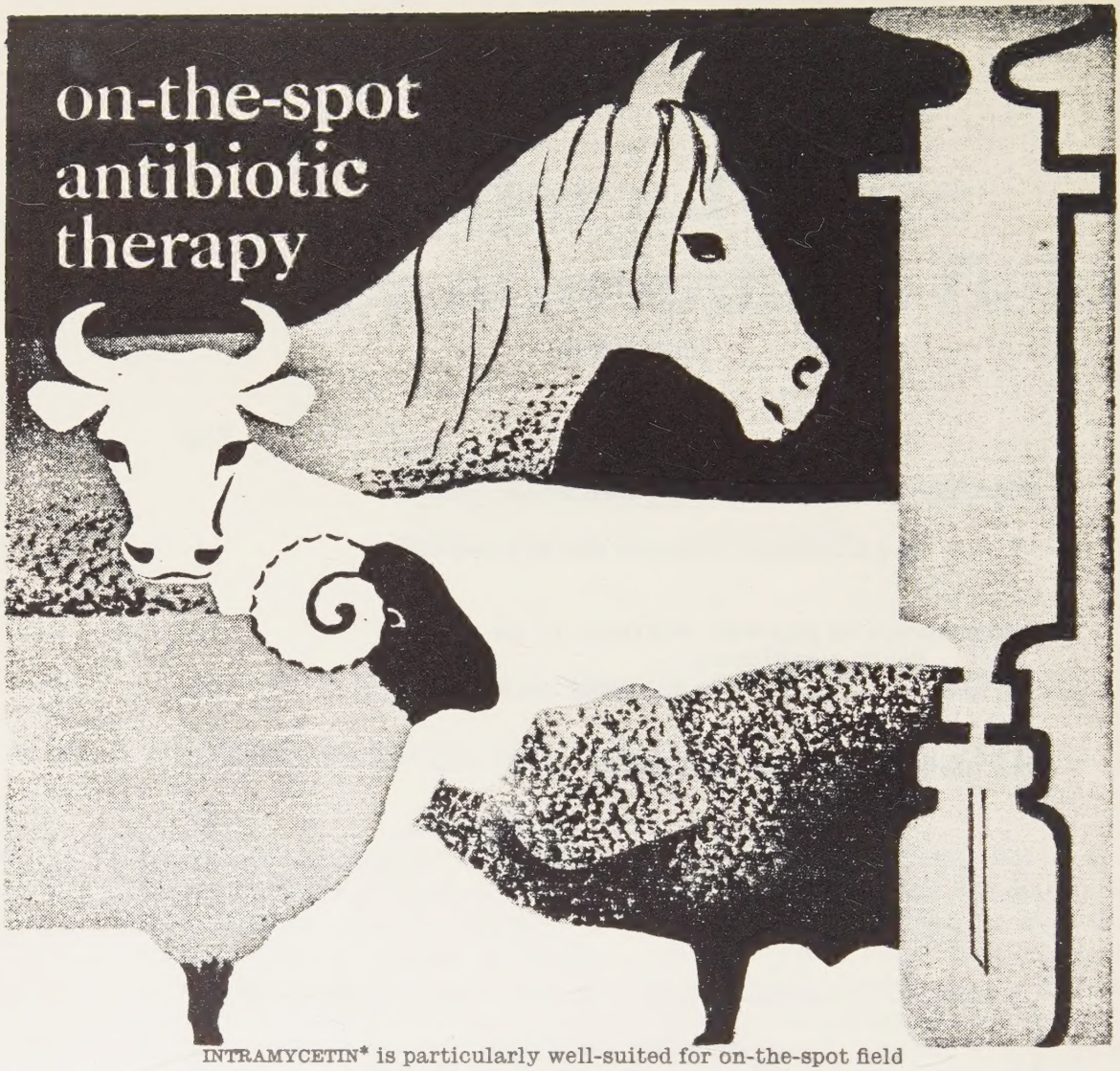
(a) The diagrams in the article on Ketosis by P.J.Pearce should be amended as follows:-

p. 59. Figure 1. Delete arrows between glycerol and lactate.
Delete asterisked footnote.
Complete reversible arrows between pyruvate and lactate.

p. 70. Figure 4. Insert reversible arrows between pyruvate and oxaloacetate (reaction 4).
Insert single arrow between pyruvate and acetyl CoA.
Delete arrow between lactate and acetyl CoA.

p. 83. Conclusion: line 1. For "metabolic" read "enzymic".

(b) page 127, line 40. The reference to Hudson, J. R. (1959) is incorrectly given. It should read:-
HUDSON, J. R. (1959). In "Infectious Diseases of Animals", Vol. 2. (Stableforth, A.W. & Galloway, I.A., edited by) (London: Butterworths), p. 415.



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COMMONWEALTH BUREAU OF ANIMAL HEALTH

VETERINARY REVIEWS AND ANNOTATIONS

Volume 7 **April, 1961** *Part 1*

Microbiological Aspects of *Corynebacterium renale*

BY

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INTRODUCTION

Corynebacterium renale causes specific cystitis and pyelonephritis in cattle, but other animals are also naturally affected, for example: dogs (Olafson, 1930), horses (Foss, 1944; Boyd & Bishop, 1937), goats (Nandi, 1955), pigs (Weidlich, 1954), and sheep (Boyd, 1918; Boyd et al., 1918; Allen et al., 1959). Both the disease and the causative organism have been known for some time. Earlier work was concerned with the nature of the organism and the pathology of the disease, and little success was achieved in the direction of therapeutics. The work of Jones & Little (1925, 1926, 1930) recognized the organism, its associated disease, and made some attempt to explain the epidemiology. Little further was done before the use of antibiotics and the work of Morse (1948a, b, 1949, 1950a, b), Morse & Wipf (1951), Morse et al. (1950), Feenstra & Thorp (1946) and Feenstra et al. (1945, 1948a, b, 1949). Some biochemical studies on the metabolism of the organism and the relationship to pathogenicity have been made also by Lovell and his colleagues (Lovell, 1946, 1951, 1953a, b, 1956; Lovell & Cotchin, 1946, 1947, 1952; Lovell & Harvey, 1950) and by Lister (1956a, b).

Most of the work on the organism appears to be incomplete. No

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proven difference has been found between pathogenic strains and those found in healthy animals as part of the normal vaginal flora. The method of infection whether haematogenous or ascending is still in doubt although it is known to be haematogenous in mice (Lovell & Cotchin, 1946). Explanations as to why the disease is more frequent in the cow rest mainly on the fact that the cow has a shorter urinary tract liable to small traumata at parturition, particularly if dystokia is involved. This could allow a focus of infection to be set up. The relation to mastitis, retention of the placenta or winter conditions is not fully understood. The pathogenesis is not truly known but is believed to be due to the destructive effects of the ammonia produced by the powerful urease the organism possesses. This is the majority viewpoint although little seems to be known about the effect of ammonia on body tissues (Bessman, personal communication). Lovell (1956) has produced some interesting effects on the kidney using extracts of the organism, but the interrelationship between the effect of this extract and the effect of the whole organism has still to be evaluated. It is well known that when the organism reaches the kidney it causes pyelonephritis, but the features of interest are: how does it reach the kidney; does it cause a shunt of blood through the medulla to set up a focus of infection; is it the urease alone that is responsible for the lesions; is the organism transmitted from animal to animal, and if so how is this done; and how important is the disease in view of its insidious nature and its tendency to subside with or without treatment? In most cases, unless the cow is very valuable it is slaughtered.

THE NATURE OF THE ORGANISM

Corynebacterium renale was first placed in the genus Corynebacterium newly formulated by Lehmann and Neumann, by Ernst in 1905 (see Ritzenthaler, 1910a, b). Earlier authors gave the organism many names. It was called Bacillus pyelonephritis bovis by Enderlen (1891) and also in the same year Bollinger isolated an organism which he called Bacillus renalis bovis. In 1893 Lucet isolated Bacillus pyogenes bovis (now Corynebacterium pyogenes), which was very similar to C. renale, and some confusion must then have existed in the identification and nomenclature of corynebacteria from cattle; Ernst (1905) had called it Corynebacterium renalis bovis, a name maintained until 1925 when Jones & Little substituted Corynebacterium renale, which is maintained in the most recent 7th edition of Bergey's Manual (1957).

Morphology: Descriptions are given by Gair (1918), Jones & Little (1925), Merchant (1935), Feenstra et al. (1945), Lovell (1946) and Dhanda & Das (1954–55). All agree that the organism is a bacillus with blunt or pointed ends, 2–3μ x 0.7μ in size, and that it is non-motile, non-capsulated and non-sporing. It exhibits metachromatic granules, bi-polar staining, pleomorphism and, rarely, branching. It is Gram-positive and appears as palisades, curved pairs or in large clumps.

Colonial morphology: This is described by Jones & Little (1925), Lovell (1946), Morse (1948a, 1949, 1950a), Ovejero del Agua (1951) and Dhanda & Das (1954–55). Most of these authors agree that the colonies are

punctiform, entire, circular, are not firmly fixed to the growth substrate, and are variable in colour. Younger types appear greyish-white and moist, whereas aged colonies are yellowish. Colonies are non-haemolytic and do not liquefy serum agar. Colony size is variable but generally not more than 0.5 mm. in diameter.

Growth in broth: The organism forms a granular deposit in young cultures but yellow and viscid in older ones. The broth may be clear or turbid and a pellicle may or may not be formed.

Sugar fermentation: The organism is not powerfully fermentative. Usually the only sugar fermented is glucose but Thorp et al. (1943) found fermentation of arabinose, dextrin, galactose, lactose, maltose and xylose. Feenstra et al. (1945) also found fermentation of laevulose and mannose.

General biochemical characters: Gelatin is not hydrolysed and growth on this substrate is described as good to poor.

In litmus milk characteristic changes occur, the milk becomes more alkaline and, as the casein is digested, the colour becomes a deeper blue; as a corollary to these results, Luzzio & Harris (1955) found casein to be the only protein attacked by 7 strains of C. renale. Nitrates are not usually reduced to nitrites but strains have been found performing this reaction (Dhanda & Das, 1954–55). Strains may vary in their reaction to the Voges-Proskauer/Methyl Red tests. Fat is not hydrolysed but urea, arginine and sodium hippurate are. Hydrogen sulphide and indole are not formed and a white halo is formed round colonies on milk agar.

Growth is aerobic or facultatively anaerobic and occurs optimally at 37°C. Some strains exhibit atypical characters, however, as exemplified by a strain isolated by Morse & Wipf (1951) which fermented trehalose and xylose, reduced nitrates to nitrites and required blood or serum for growth.

THE NATURE OF THE DISEASE

This is essentially similar in all species and, since cattle are the most important, the description will be limited to this species. Ritzenthaler (1910a, b) admirably sums up all earlier work, and descriptions by modern authors differ only slightly from descriptions quoted in his papers. Later authors describing the disease, or aspects of it, in detail are: Lovell (1951, 1953a, b), Thorp et al. (1943), Feenstra & Thorp (1946). The disease is diagnosed in the earliest stages by the passage of bloody, ammoniacal urine with signs of renal colic and obvious pain in the animal. Isolation and cultivation of diphtheroids from the urine is a diagnostic feature in combination with the above. General debility and, rarely, death may follow immediately but slow recovery and relapse is most usual. At P.M. examination the kidneys are much enlarged. The pelvis and surface of the kidney have many necrotic foci. The capsule is adherent and the dilated pelvis full of muco-purulent fluid smelling of ammonia and containing leucocytes, bacteria and crystals of magnesium ammonium phosphate. The bladder is contracted and the ureters thickened and fleshy. Lesions have also been noticed in other parts of the body (Arthur, 1949) but these may or may not be coincidental. Foetid urine may be retained in the thickened, shrunken bladder. This descrip-

tion of the gross pathology is fairly constant and is found in advanced cases. The infection may be uni- or bi-lateral.

The histopathology has been extensively reviewed from 1905 onwards. The general description consists of three stages:

(1) A stage of infection in the early phases with extensive invasive lesions in the kidney. Bacteria mass in the tubules and are excreted in the urine. Tissue debris blocks the medullary tubules giving them a striped aspect. The mucosa of the papillary tubules is infiltrated by leucocytes and multiple discrete abscesses are formed on the kidney surface. Erythrocytes appear in the urine and kidney tubules. Feenstra & Thorp (1946) noted in addition, necrosis wherever living bacteria occurred in the kidney, which they thought due to a diffusible toxin such as is produced by the diphtheria organism, and also haemorrhages in the walls of the urinary bladder.

(2) In this stage the disease has progressed and the kidney reacts by extensive leucocyte infiltration of extending lesions in the kidney tissue. These lesions thus tend to become localized and much infiltration occurs in the medullary region.

(3) This is the stage of recovery where scar tissue replaces necrotic and also functional kidney tissue. This is a healing stage and the scar tissue tends to obliterate the normal kidney structure. The papillae are converted into a mass of fibrous tissue. Connective tissue strands may push between glomeruli and convoluted tubules and substitute for normal kidney elements. If the destruction is extensive as in long relapsing cases a spongy cavernous pyelonephritis may occur with destruction of kidney function. This description is mainly that of Ritzenthaler (1910a, b) but those of later authors show little variation.

Diagnosis is possible only when the disease is in a fairly advanced state and when destruction of kidney tissue has occurred. In other animals the pathology is essentially similar. In pigs, however, the disease is caused by an organism tentatively named Corynebacterium suis by Soltys & Spratling (1957), the biochemical and other characters of which indicate a very close relationship to C. renale.

Much work has been done also on experimental animals and the pathology in these cases has been described by Jones & Little (1930), Feenstra et al. (1945, 1948b) and Morse & Wipf (1951).

Epidemiology: This is somewhat controversial. Jones & Little (1925, 1926) were the first to study this aspect. They tried to assess the distribution of the organism in normal animals but failed to do so. They found that the disease was not transmitted by coitus. Proximity of cows and washing and brushing with the same implements were conducive to spread in a stabled herd. Recently calved cows were particularly susceptible, since the increased flow of urine and its high nitrogen content resulting from a high protein diet provided a suitable environment for the organism. At parturition minor injuries from dystokia may allow infection by organisms of the normal vaginal flora. They demonstrated early post-natal infection by finding the disease in a 12-day-old calf (heifer). Not all strains isolated from calves were pathogenic. Inoculation experiments suggested that the ascending mode of infection was possibly predominant. In later work Morgan et al. (1946) showed the growth of

Trichomonas foetus to be enhanced by the presence of C. renale but the role of the latter is unknown. These authors considered that copulation may transmit the disease because they obtained high diphtheroid counts (10×10^6 organisms/ml.) from bull sheath scrapings. Weitz (1947) demonstrated that the organism may be a normal inhabitant of the bovine vagina with infection due to certain ill-defined conditions. Transmission from diseased to healthy animals he believed to be unnecessary since many or most animals carry the organism in the vagina. Morse (1950b) found proximity of stabled cows to be a major factor in transmission. Hartwigk (1959) found C. renale in two semen samples from one bull and in two biopsy samples of endometrium from a cow.

THERAPY

Under this heading both antibiotic sensitivity and treatment in vivo may be considered.

(a) Antibiotic sensitivity. Beck et al. (1945) reported on the sensitivity of C. renale to penicillin, at 0.025 unit/ml in broth. Lovell & Cotchin (1947) found a sensitivity range of 0.1–0.0001 unit/ml., indicating an extremely high in vitro sensitivity to penicillin. Morse (1948a, b) obtained values ranging from 0.0039–0.312 unit/ml. for 43 strains. Morse et al. (1950) found 2.5 μg. of oxytetracycline were needed for 24 hour inhibition and 2–8 μg./ml. for 48 hour inhibition. Ovejero del Agua (1951) found that 0.1 unit/ml. penicillin gave satisfactory inhibition. Larson et al. (1954) studied the in vitro sensitivity of C. renale to seven major antibiotics. They concluded that penicillin was effective for most strains at 0.5 unit/ml.; that bacitracin inhibited the majority at 20 units/ml., while chloramphenicol inhibition ranged from 10–50 μg./ml., with oxytetracycline at 60 μg./ml. and chlortetracycline inhibiting at 10 μg./ml. Polymyxin B was the least effective and gave poor inhibition even at 20 μg./ml. The authors considered it noteworthy that four strains were not inhibited by the lower levels of penicillin—a true resistance since the organisms had been isolated prior to widespread use of this antibiotic—and this led them to suggest large doses of penicillin in the actual treatment of the disease. Frost et al. (1955–56), using cathomycin (novobiocin), found 0.39 μg./ml. to be inhibitory for 24 hours. They included also the sensitivity to penicillin G and erythromycin at 0.094 μg./ml. and 0.024 μg./ml. respectively.

(b) Treatment in vivo. Early methods aimed at increasing the acidity of the urine to combat infection and Gair (1918), for example, gave a cow boric acid, gruel and turpentine and the disease resolved in three months. Boyd (1927) practised nephrectomy which was sometimes successful in unilateral infections. Olney (1943) treated a cow with calcium borogluconate, large volumes of water and blood transfusions for 16 days but death resulted.

Antibiotics radically altered methods of treatment but were not always successful. Beck et al. (1945) found that 10×10^6 units of penicillin alleviated symptoms temporarily although frequent relapses

occurred. They concluded that sodium-penicillin was useful in treatment although relapse and spontaneous recovery were frequent. Fox (1947) found that oil or beeswax suspensions of penicillin gave better results than aqueous suspensions. He considered penicillin to be useful in prolonging the life of a valuable animal. Kiesel et al. (1947) used penicillin in a beeswax-peanut oil base and administered 10–15 million units in seven days. Out of seven cows treated four recovered, but one was slaughtered and another had a serious relapse. Another of the four made doubtful recovery since diphtheroids were isolated from the urine on two out of five examinations. The authors indicated that the health of cows that have apparently recovered needs frequent inspection particularly in the winter and at parturition to assess the efficiency of treatment. They thought that sulphonamides could also be used with penicillin to treat valuable animals. Morse (1948b) found that subcutaneous injection of 200,000 units of penicillin gave satisfactory concentrations in the blood, plasma and urine. Oil suspensions were more efficacious than aqueous. Morse believed that antibiotic therapy might salvage a cow for a short period but slaughter was ultimately necessary. Arthur (1949) gives an extensive account of penicillin therapy. Clinical recovery occurred except where the disease was severely chronic. He used daily doses of 100,000–500,000 units/ml. over a few days to a total of 5–10 x 10^6 units. Merriman (1949) suggested less frequent treatment. One million units of procaine penicillin every 48 hours would, he believed, give a more satisfactory level than smaller more frequent doses. Morse (1950a, b) insisted on the synergy of sulphonamides and penicillin and 2–3 million units over ten days was recommended. Thompson (1951) claimed spectacular results using, e.g. doses of NaH_2PO_4 to acidify the urine combined with two million units of aqueous penicillin G. Roberts (1953–54) thought that 3 x 10^6 units of aqueous procaine penicillin with 3 x 10^6 units of dibenzylethyldiamine G penicillin injected intramuscularly followed by the same dose five days later would be sufficient to clear most cases of infection. Jones (1954) considered sulphonamides to be the logical therapeutic agent since they are excreted in high concentration in the urine: sulphathiazole, for example, attains a high concentration in the urine with a low oral dose but he considered Gantrisin (sulphisoxazole) to be the most effective. A very high urinary concentration is attained some 5–6 hours after injection which is not reached if the drug is administered orally. Sulphisoxazole is also recommended for the treatment of calves since it is more soluble in acid or neutral urine. Smrček (1957) used one million units of penicillin/day, in combination with trypaflavine, for ten days. Sulphonamides or trypaflavine alone had no effect on the disease. Kwiatkowski (1957) described treatment of two cases. One cow was given streptomycin, sulphonamides, diuretics, urinary antiseptics and trace elements and died on the eleventh day of treatment. The other cow was sent for slaughter. He considered treatment valueless once the disease has damaged the kidney irreparably.

BIOCHEMICAL ASPECTS

The most important factor in relation to the pathogenicity of

C. renale appears to be its powerful urease. The activity of this enzyme was noted very early in studies of the organism. Ernst (1905) noted the strongly ammoniacal odour from the urine of infected cows. He also found that the addition of urine to nutrient gelatin greatly enhanced the growth of the organism. Ritzenthaler (1910) had suggested that the ammonia in the urine was produced by a specific "ferment" produced by the bacterium. He believed ammonia to be an important factor in pathogenesis and the urea of urine to be an important bacterial nutrient. The ammonia irritated the kidney and caused leucocyte infiltration in advance of the major lesions. Also the number of bacteria present in necrotic foci was small in relation to the amount of leucocytic infiltration produced. He also observed that bacteria died out when scar tissue replaced functional kidney units.

An interval of 40 years followed with very little mention of the subject until 1950 when Lovell & Harvey at the Royal Veterinary College worked on the urease, and other enzymes, of C. renale and compared it with that from other organisms, namely: C. ovis, C. equi, and C. pyogenes. They observed that urease accounted for the bulk of ammonia production in C. renale. An uncharacterized arginase and amino-acid deaminases were also found. The authors speculated on the possibility of base formation in the kidney by the action of amino-acid decarboxylases as occurs in E. coli. No such bases have been demonstrated for C. renale. Lister (1956a) worked solely on urease as a biochemical problem. He found a qNH_3 value (μg. NH_3/mg. dry wt. org.) of 20 x 10^3, higher than catalase activity in some organisms. He found it as a constitutive enzyme and fully discusses the kinetics. The urease was rapidly inactivated by oxygen in cell-free extracts. The urease differed little from that from other sources. It had, however, a wider pH range and was very active at any physiological pH likely to be encountered by the organisms. The enzyme accounted for about 0.1% of the bacterial cell. Kinetic evidence gave no account of the role of the enzyme in the aetiology of the disease.

Lister (1956b) also found that up to 40% of the nitrogen assimilated by the organism could be derived from added labelled urea or ammonia. Lovell (1956) worked on a cnemical extract prepared from the organism. He did some serological work which indicated the passage of antibodies from mother to calf in the colostrum. Two peaks of antibody titre were demonstrated, one in the calf and one in the cow. Injections of the extract into the kidneys of mice produced a cortical ischaemia similar to that noted by Trueta et al. (1947) using rabbit kidneys and staphylococcal toxin. A mechanism similar to this would explain the localization of the organism in the kidney medulla after primary infection. The shunting of the blood from the cortex would ensure a high enough concentration of organisms in the medulla to be actively invasive. The observations on antibody production give weight to the suggestion that C. renale is a normal bovine vaginal inhabitant.

ECONOMIC IMPORTANCE

Although the disease is well known in most European countries and

America, and has for example only recently been discovered to be prevalent in Australia according to Carne (personal communication, 1958), there are no economic data available for the disease. The Commonwealth Bureau of Animal Health (personal communication) indicated that the incidence of the disease did not seem to be high enough in any country to have warranted the making of surveys.

Acknowledgments. The authors would like to express their thanks and appreciation to the Commonwealth Bureau of Animal Health, and to Miss N. W. Rhodes and her library colleagues of the University of Nottingham School of Agriculture for ready help on the source material. One of us (M. J. C.) wishes to express his gratitude to the Preston Borough Council for a grant during the tenure of which this work was performed.

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Veterinary Reviews and Annotations
Vol. 7, Pt. 1 (April 1961)

Salmonellosis in Pigs with reference to its Public Health Significance

BY

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INTRODUCTION

Pigs and poultry are the biggest reservoir of salmonella infection in the animal kingdom (Edwards, 1956; Buxton, 1957; Gaugusch, 1958). Of the salmonella serotypes which have so far been described (over 600), most have a very wide zoological distribution, others show a degree of host specificity, but all are regarded as at least potentially pathogenic for man or animals, or both. The pig is a natural host of S. cholerae-suis which is mainly known for its association with porcine paratyphoid, but it has also been found in man, turkeys, chicks, canaries, cattle, horses, sheep, dogs, foxes, a cat, mink and guinea-pigs (Edwards et al., 1948). This serotype was the first of the paratyphoid organisms to be isolated, in 1885 (Salmon, 1886; Salmon & Smith, 1886) and since then a wide variety of Salmonella serotypes has been recovered from pigs, especially from the mesenteric lymph nodes, but it is generally considered that S. cholerae-suis is the most important cause of clinical salmonellosis especially in pigs between the ages of two and four months (Buxton, 1957; Field, 1958; Sojka, unpublished, 1959).

THE INCIDENCE OF SALMONELLOSIS IN PIGS

A. Diseased pigs

S. cholerae-suis is considered to be the most common cause of clinical salmonellosis in Britain (Field, 1958). Buxton (1958) has stated that the majority of clinical infections in pigs are due either to this serotype or to S. typhi-murium, but many other serotypes have been isolated on occasion.

A survey of causes of mortality in pigs carried out by the Veterinary Investigation Service in England and Wales during the period 1956–58, listed S. cholerae-suis as one of the most important bacterial infections in pigs 2–4 months old (Anon., 1959). Its invasive properties in the pig were attested by its frequent isolation from the blood and parenchymatous organs, whereas the other serotypes were usually confined to the intestine and mesenteric lymph nodes (Edwards et al., 1948; Field, 1959).

At the Weybridge Veterinary Investigation Centre, S. cholerae-suis was recovered from 23 (14.6%) of 157 batches of pigs aged 2–4 months, received for diagnosis between March 1957 and September 1958 but it could not be found in 633 batches of pigs of other ages received at the same time (Gitter, 1959).

From 1st January 1958, records of all the isolations of salmonella at seventeen V. I. Centres in England and Wales were collected at the Central Veterinary Laboratory, Weybridge. Up to that date, isolates of most of the rarer serotypes were sent to Weybridge for typing, whereas those of the common serotypes were sometimes typed at the local Centre and not reported. The incidence of the different serotypes in pigs is shown in Table 1, and indicates that S. cholerae-suis is the type recovered most commonly from diseased pigs. The majority of the strains are monophasic and produce H_2S, i. e., they are S. cholerae-suis var. kunzendorf. The data for 1958 and 1959 are probably representative of the country as a whole since they include all isolations at V. I. Centres in England and Wales.

The case histories which were received with the cultures indicated that S. cholerae-suis and S. typhi-murium were associated with clinical disease while other serotypes were probably coincidental.

Table 1

Distribution of Salmonella types isolated from diseased pigs

Salmonella types	Strains isolated at V.I. Centres and typed at Weybridge 1953–1957	Incidents reported by V.I. Centres in England and Wales	
		1958	1959
1. S. cholerae-suis	121(71%)	72(87.7%)	90(90.9%)
2. S. typhi-murium	20(12%)	6(7.5%)	6(6.7%)
3. S. agam	-	1	1
4. S. anatum	1	-	-
5. S. bovis-morbificans	2	-	-
6. S. brandenburg	-	-	1
7. S. dublin	10	2	-
8. S. enteritidis	6	-	-
9. S. gallinarum	3	-	-
10. S. infantis	1	-	-
11. S. livingstone	-	-	1
12. S. meleagridis	1	-	-
13. S. muenchen	-	1	-
14. S. pullorum	2	-	-
15. S. taxony	1	-	-
16. S. thompson	2	-	-
Total	170	82	99

Workers in other countries have also found S. cholerae-suis to be that most commonly associated with clinical salmonellosis in pigs.

Salisbury (1958) regarded salmonellosis as the most serious disease of pigs in New Zealand and found S. cholerae-suis to be most commonly involved. Of 416 salmonella strains isolated from pigs during 1948-57, 407 were of this serotype; the other strains were S. typhi-murium (8) and S. senftenberg (1).

Simmons & Sutherland (1950) reported that salmonella was found more often in pigs than in other domestic animals in Queensland, Australia, and that S. cholerae-suis was the commonest type.

In Germany, Lütje (1938) isolated salmonella from 235 (3%) of 7,778 pigs in 15 years; 187 strains were S. cholerae-suis.

In Argentina, Quiroga & Monteverde (1945) reported that S. cholerae-suis was the dominant serotype in diseased pigs, but not in healthy pigs.

In U.S.A., Levine et al. (1945) examined 194 salmonella strains isolated from diseased pigs, and found 137 (69%) to be S. cholerae-suis, 16 S. typhi-murium and 15 S. bredeney. Eleven other serotypes were identified (1-7 strains of each). S. cholerae-suis was the only organism which these authors isolated consistently from the viscera, and they suggested that it might be the only one with invasive power while other salmonella serotypes could be normal to the digestive tract of healthy pigs. Edwards et al. (1948) reported that salmonella cultures isolated from pigs were greater in number than those from any other species, except for man and fowls. S. cholerae-suis comprised 906 (44%) of 2,060 cultures examined. This serotype constituted approximately 95% of cultures from blood and parenchymatous organs, while the majority of other serotypes were isolated from intestine and mesenteric lymph nodes. These authors listed a total of 37 different serotypes isolated from pigs. Genev & Slavkov (1956a) in Bulgaria, reported that of 365 strains of salmonella isolated from outbreaks of swine paratyphoid, 362 were S. cholerae-suis. Zhekov & Savov (1956) in a similar investigation isolated 124 strains from diseased pigs of which 117 were S. cholerae-suis var. kunzendorf. Žakula (1956) in Yugoslavia reported isolation of S. cholerae-suis var. kunzendorf on 26 occasions from 101 sick pigs examined. Steffen & Szaflarski (1959) reported that during 1955-57 in one district of Poland (Katowice), 1,145 pigs were found to be infected with salmonella, and that the majority of the infected animals (908) were encountered in 1957. The strains isolated from dead pigs were S. cholerae-suis. Most of the isolations were from pigs infected at fattening centres.

B. Apparently healthy pigs

1. Mesenteric lymph nodes. Hormaeche & Salsamendi (1936, 1939) were the first to carry out a systematic examination of mesenteric lymph nodes of apparently healthy pigs, slaughtered for market, and they showed how commonly these animals carried salmonella. In the first series of observations (1936) they collected and pooled the mesenteric lymph nodes from 20 pigs in each of 46 lots, 22 of which (47.9%) yielded eleven different types of salmonella. In further investigations (1939) 32 of 37 similar lots yielded nine types of salmonella and of 14 lots consisting of 2-5 pigs, eight yielded seven types. When they examined

mesenteric lymph nodes from individual pigs 16 (64%) yielded eight types of salmonella. On the other hand in 19 pigs from which the nodes were taken aseptically, only four (21%) were positive. The 1936 and 1939 investigations together yielded a total of fifteen serotypes, but only one strain was S. cholerae-suis.

The presence of a variety of salmonella types in mesenteric lymph nodes of normal pigs, was confirmed by many workers, most of whom found that S. cholerae-suis was not the predominating type.

Varela & Zozaya (1941) in Mexico, recovered salmonella on fifteen occasions from 74 sets of mesenteric lymph nodes taken from a total of 209 healthy pigs; one of these strains was S. cholerae-suis.

In Denmark, Momberg-Jørgensen (1944) examined mesenteric lymph nodes from 3,660 pigs and found salmonella only on two occasions (S. typhi-murium and S. enteritidis var. danysz).

In Holland, Clarenburg et al. (1949) isolated salmonella from mesenteric lymph nodes of 14 (2.8%) of 503 pigs examined; none of these isolates was S. cholerae-suis.

In France, Buttiaux et al. (1951) examining similar material (147 lymph nodes), recovered salmonella on six occasions.

In Java, Kraneveld et al. (1951) examined 150 healthy pigs and isolated salmonella from the mesenteric lymph nodes of 25.

Utojo (1953) in Indonesia, recovered 17 strains from mesenteric lymph nodes of 388 pigs slaughtered in abattoirs of Bogor and Djakarta.

Fournier et al. (1953) in Saigon, isolated 35 strains (23 of which were S. cholerae-suis) from a similar material from 360 pigs.

In U. S. A., Edwards et al. (1948) examined 2,060 salmonella cultures from pigs and found 37 different serotypes. They pointed out that the majority of cultures from mesenteric lymph nodes were not S. cholerae-suis. Rubin et al. (1942) isolated 13 different serotypes from 19 (47.5%) of 40 lots of pooled nodes (each lot taken from 25 healthy pigs). Of 50 pigs examined individually, five yielded salmonella.

In Yugoslavia, Žakula (1956) isolated S. cholerae-suis var. kunzendorf from the mesenteric lymph nodes of two of 240 healthy pigs.

In Norway, Bøvre (1957) examined mesenteric lymph nodes of 4,114 pigs brought for slaughter in the Oslo abattoir. Of 436 pooled samples 45 (10.3%) yielded salmonella.

In England, Scott (1940), who examined bacteriologically mesenteric lymph nodes and spleens from healthy slaughtered pigs at two large bacon factories, found salmonella in 20 of 50 batches, each of 10 pigs, in the factory in the West of England, and in 16 of 50 similar batches in the factory in East Anglia. In each factory one lot yielded two different serotypes. Of a total of 38 strains isolated from 1,000 pigs, 10 were S. cholerae-suis. Similar investigation in 1944–45 (Report, 1947) yielded 133 (2.5%) isolates from 5,285 pigs. There were 18 different serotypes. The commonest, S. typhi-murium, was isolated 37 times, but S. cholerae-suis only once. Report (1955) gave even lower figures; from 409 samples of mesenteric lymph nodes only one strain (S. typhi-murium) was isolated. Smith (1959), using brilliant green MacConkey broth, isolated salmonella from mesenteric lymph nodes of 60 out of 500 pigs examined (12%). The most common serotype was S. typhi-murium (17) followed by S. anatum (16),

and S. cholerae-suis (11); 14 other serotypes were also isolated.

2. Faecal material. In the U.S.A., Levine et al. (1945) isolated seven salmonella strains, none of them S. cholerae-suis, from the faeces of pigs. Galton et al. (1945a) isolated salmonella from 1,476 out of 2,822 samples of faeces taken from pigs at an abattoir in Florida. There were 36 serotypes, of which the commonest was S. anatum (547 strains), followed by S. derby (539), S. bredeney (52), S. newport (47) and S. typhi-murium (38). S. cholerae-suis was isolated only once. There was an appreciable difference in the proportion of infected pigs on farms and in the abattoir. Of 374 anal swabs taken from live pigs on 28 farms, only 27 (7.2%) were positive for salmonella, and 18 of these were from 34 animals on two farms where the pigs had acute diarrhoea.

In Germany, Schaal (1957) reported that 5% of faecal samples taken from healthy pigs were infected with 11 different salmonella types.

Clarenburg et al. (1949) in Holland, failed to find salmonella in the faeces of 503 pigs.

In the United Kingdom, Smith & Buxton (1951) isolated four salmonella strains from faeces of 600 healthy pigs; three were S. meleagridis and one S. typhi-murium. Smith (1959) found six (1.2%) salmonella excretors among 500 normal pigs, five isolates being S. typhi-murium and two S. anatum. In another survey (Report, 1955), no salmonella was recovered from 677 samples of faeces from healthy pigs.

McDonagh & Smith (1958) carried out a similar investigation in Bradford abattoir. Rectal swabs of 171, taken immediately after the animals arrived at the lairage showed that 2.9% of pigs were excreting salmonella organisms, whereas bacteriological examination of animals maintained from one to seven days (or longer) in the lairage showed that 13.5% were harbouring this organism, (36% of which were S. typhi-murium and 64% S. derby). Human salmonellosis in the area at that time was closely related to the abattoir findings.

Newell et al. (1959) in Northern Ireland, isolated 23 salmonella strains from 100 samples of caecal contents and from 9% of rectal swabs from 162 pigs on five farms.

The isolation of salmonella from healthy slaughtered pigs may be only a reflexion of the environment in which the pigs were kept before slaughter. It is well known that such an environment is often heavily contaminated with a wide range of salmonella. For reliable figures on carrier rates it would be necessary to examine pigs slaughtered away from a contaminated environment which would be difficult.

THE EXPERIMENTAL REPRODUCTION OF SALMONELLOSIS IN PIGS

For many years it was believed that necrotic enteritis in pigs, caused by S. cholerae-suis, could not exist independently but was associated with swine fever. When in 1885, Salmon (1886) described S. cholerae-suis, he believed that this organism was the cause of swine fever (hog cholera) and in 1889 he reproduced experimentally a bacterial disease somewhat resembling swine fever but which did not spread naturally or easily to susceptible pigs. It was found later that the causal agent of swine fever is a virus, but it was not until Murray and his co-workers in

1927 and 1929 showed that S. cholerae-suis was the primary aetiological agent in infectious enteritis, that it was proved experimentally that salmonellosis of young pigs can exist as an independent disease. Biester (1934) stated that S. cholerae-suis is capable of producing a pathogenic enteric condition under poor sanitary conditions in the field in both cholera-immune and susceptible pigs and that it can be produced experimentally.

A number of workers have since reported the successful experimental reproduction of salmonellosis, usually by feeding large quantities of S. cholerae-suis cultures to susceptible pigs (Shanks & Lamont, 1938; Schofield, 1944; Levine et al., 1945; Guthrie, 1952; Josland, 1954; Kernkamp & Lindorfer, 1959, and others).

Slavin (1942) set up infection in newly weaned pigs by turning them onto pasture sprayed recently with a culture of S. cholerae-suis. He found that pigs could be infected by contact, during the early stages of an outbreak, by placing them on ground vacated a few hours previously by recovered pigs. In later experiments, Slavin (1951) found that pigs could regularly be infected with a comparatively small number of S. cholerae-suis organism. Even a dose as small as 0.0005 ml. of culture was sufficient to infect the pig (although only one pig from this group had diarrhoea, there was a general loss of condition). In his experiments groups of 5–10 pigs aged 8–12 weeks were infected orally with approximately 200,000 to 400,000,000 S. cholerae-suis organisms, and although the mortality was low, the disease produced was similar to that seen in the field.

Levine et al. (1945) also demonstrated the pathogenicity of S. typhimurium, by feeding large quantities of cultures to susceptible pigs.

CLINICAL MANIFESTATIONS

All pigs are susceptible to paratyphoid but young pigs from five or six weeks to four months are most commonly affected. Although the influence of various stress factors like errors in management, feeding and housing, parasitism, concurrent infections, etc., can appreciably lower the pigs' resistance to infection, outbreaks can occur in well managed pigs free from other infections.

The incubation period varies from two days to several weeks (Buxton, 1957) and clinical symptoms vary from very acute, septicaemic disease, to a chronic, debilitating condition. In the acute form, a high temperature, inappetence and red or purple discoloration of the skin are often noted on the extremities, particularly on the ears, and also on the abdomen and thighs. Death may occur within 1–4 days from the onset of symptoms, though outbreaks have been seen where the first one or two pigs were found dead without any of the premonitory symptoms, which were noted later in the in-contact animals. Diarrhoea may develop but it is not a constant feature of the septicaemic form of the disease. Spontaneous recovery is rare and when no efforts at control are made, emaciation, sometimes ataxia, usually in the scouring animals, prostration and death follow frequently.

Post-mortem examination reveals a septicaemic carcass. The

spleen, liver and kidneys are generally hyperaemic and the spleen often enlarged; petechial haemorrhages are found frequently in the mucous membrane of the stomach and intestines, in the kidneys, lungs and epicardium. The carcass and mesenteric lymph nodes are enlarged and haemorrhagic. The gastro-intestinal mucous membranes vary considerably from apparently normal to intensely congested, and since gastritis or enteritis, or both, are a feature of many diseases of pigs, their presence is of no diagnostic significance.

Pigs affected with the sub-acute or chronic form of the disease sometimes referred to as enteric paratyphoid (Report on Diseases of Farm Livestock, 1947) show a slight to moderate rise in temperature, dullness, poor appetite, diarrhoea, staring coat and loss of condition, which may lead to severe emaciation and death. Yellowish faeces, having an offensive odour, are often reported (Buxton, 1957). Animals which recover clinically are seldom profitable, and often, after a temporary recovery, develop necrotic enteritis. Post-mortem examination reveals emaciated carcass, with rough, long hair and although the lesions may vary considerably in intensity, they are usually confined to the gastro-intestinal tract. The gastric mucosa may, in parts, be congested, and even show some sloughing. The mucous membrane of the small intestine may be oedematous, moderately congested and covered with catarrhal exudate, or sometimes may even show slight evidence of diphtheresis. The main lesions are usually seen in the caecum and colon, where the walls are thickened and the mucous membrane covered with a yellowish green to grey layer of necrotic tissue, which may be sloughed and mixed with intestinal contents. These lesions vary in size and extend from small, circular or irregular spots, to extensive areas involving the caecum, colon, and sometimes the last foot or two of the small intestine, with a soft, thin, diphtheritic membrane or a thick, caseous deposit. These deposits of necrotic or caseated tissue can be detached easily, leaving underneath a red, diffuse surface, and sometimes irregular black patches. The mesenteric lymph nodes, especially those associated with the large intestine, are enlarged, oedematous, and sometimes moderately congested.

A "latent" S. cholerae-suis infection is often encountered among weaned and store pigs in herds with no history of salmonellosis or any other disease The clinical symptoms are unthriftiness, slow growth, occasional diarrhoea and sometimes death. No other symptoms are noted, the temperature remains normal and there is no inappetence. Post-mortem findings are not suggestive although catarrhal enteritis and enlargement of the mesenteric lymph nodes can be seen in a proportion of such animals; a few may show necrotic typhlitis or colitis, or both, but in a high proportion no gross abnormality can be seen, except for a poor carcass, often covered with long, rough hair.

ISOLATION OF S. CHOLERAE-SUIS

There is no difficulty in isolating the organism from the visceral organs, heart blood and mesenteric lymph nodes of pigs which have died from a salmonella septicaemia but in many cases of sub-acute, chronic

and latent infections, routine bacteriological examination is completely negative. The difficulty in isolating S. cholerae-suis from contaminated material has been reported by several workers (Slavin, 1943; Smith, 1952; Gitter, 1959). Slavin (1943) and Smith (1952) found that brilliant green agar was superior to all other media for primary isolation and Josland (1947) confirmed that the best recovery rate of S. cholerae-suis was obtained by the use of Slavin's medium. Gitter (1959) reported that when 30 strains of S. cholerae-suis were mixed with pigs' intestinal contents, and incubated for 24 hours, at 37°C., only 11 were recovered on MacConkey agar and 29 on Slavin's medium; after 48 hour incubation, seven (23.3%) were recovered on MacConkey's and 25 (83.3%) on Slavin's medium. The adoption of brilliant green–neutral red–lactose agar, and routine cultural examination of pigs' gall bladders, greatly increased the isolation of S. cholerae-suis at Weybridge Veterinary Investigation Centre from 1.33 to 12.54% in pigs 6–12 weeks old.

The results of all these investigations are difficult to interpret in view of the different techniques used. Moreover selenite medium which is unsuitable for the cultivation of S. cholerae-suis was sometimes used for primary isolation. Leifson, who originally introduced the selenite enrichment medium, wrote in 1936 about the high toxicity of selenite for S. cholerae-suis. Smith (1952) and Gitter (1959) confirmed Leifson's observations, unfortunately this peculiar behaviour of S. cholerae-suis is sometimes forgotten, probably because selenite broth is used for the isolation of salmonella from other species of animals, and man.

It is perhaps pertinent to point out here that in the "C_1O" group of Kauffman-White Salmonella Schema, there are three organisms, namely: S. cholerae-suis, S. typhi-suis, and S. paratyphi C, having an identical antigenic structure, O=6, 7; H=C↔1, 5 (S. paratyphi C may have the additional antigen "Vi"). Their differentiation is based on biochemical reactions, which are shown in Table 2. Poor growth of S. typhi-suis could be an additional help in differentiation. S. cholerae-suis var. kunzendorf is monophasic, having the "1. 5" phase only.

Table 2

Biochemical reactions of S. paratyphi C, S. typhi-suis, S. cholerae-suis and S. cholerae-suis var. kunzendorf.

	S. paratyphi C	S. typhi-suis	S. cholerae-suis	S. cholerae-suis var. kunzendorf
Dulcitol	AG	—	—	—
Mannitol	AG	—	AG	AG
Xylose	AG	AG	AG	AG
Trehalose	AG (2–3 days)	AG (1–2 days)	—	—
Arabinose	usually AG	AG	—	—
Rhamnose	AG (1–2 days)	AG	AG	AG
H_2S	+	—	—	+

AG = acid and gas produced; — = no fermentation; + = production of H_2S.

SALMONELLA AGGLUTININS IN THE SERA OF HEALTHY AND EXPERIMENTALLY INFECTED PIGS

The incidence of salmonellosis in pigs cannot be determined from agglutination tests alone. Lovell (1932) found "H" agglutinins to common salmonella in the serum of many normal pigs, of 202 pigs the sera of 190 agglutinated one or more serotypes to a titre 1:20 or above. The highest titre, 1:640 was found in two pigs, and in both the S. cholerae-suis H specific antigen.

In further studies Lovell (1934) showed that the majority of sera taken from 263 healthy pigs, agglutinated several suspensions ("O" and "H") of salmonella. The "H" suspensions were in most cases agglutinated to a higher titre than the "O" ones. Lovell suggested that the presence of these agglutinins was most probably due to stimulation by bacteria which are normally present in the intestinal tract or tissues of animals, but are not members of the salmonella group, having only some slight antigenic association with salmonella.

Hindmarsh et al. (1939) examined sera from pigs with dysentery and from pigs experimentally infected with S. cholerae-suis. In the experimental pigs only one had definite symptoms but the subclinical infections were sufficient to produce an "H" response to a titre of 1:1280 and to evoke an "O" response up to 1:640. Flagellar (H) titres above 1:200 were obtained 5–10 days after feeding S. cholerae-suis cultures. Hindmarsh considered it reasonable to expect active salmonella infection would give titres equal to, if not higher than these, according to the time elapsing since infection. Levine et al. (1945) reported that the "H" antigen agglutination titres of 110 pigs' blood samples examined, did not indicate that any of the animals were suffering or had suffered from active infection with S. cholerae-suis var. kunzendorf, S. bredeney or S. typhimurium. Most of these pigs were submitted for autopsy and approximately one-third of them showed gross lesions of the lower digestive tract.

DIFFERENTIAL DIAGNOSIS

It is sometimes difficult, on post-mortem examination, to differentiate between paratyphoid and swine fever, especially when both infections may be present in the same piggery.

The acute, septicaemic cases of both diseases often produce lesions which can be confused, and the "turkey egg" kidney haemorrhages characteristic of swine fever and congested lymph nodes, have been seen in cases of S. cholerae-suis septicaemia. In the chronic cases, the necrotic intestinal deposits in paratyphoid are very superficial, and are more readily removed than the swine fever "button ulcers" which are deep seated and generally impossible to remove without damaging the intestinal wall.

Clinical history, especially in a large piggery, may be helpful. Swine fever spreads very rapidly, and can affect pigs of all ages, while clinical paratyphoid is most common in pigs 6–14 weeks old. Isolation of salmonella does not exclude swine fever.

Histological examination of brains, and the gel diffusion precipitin

test, are of great diagnostic value, although they may not detect the peracute or very early cases of swine fever.

Nicotinic acid deficiency induces a condition similar to the chronic form of paratyphoid and "it is possible that pigs affected with this deficiency may suffer from a more severe or extended attack of paratyphoid than pigs not so affected" (Field, 1959). This deficiency can be prevented and should respond to a suitable correction of the diet, which, of course, would have little or no effect in case of S. cholerae-suis infection.

VACCINATION AND TREATMENT

1. Vaccination

Reports vary on the efficacy of vaccines in the control of salmonellosis in pigs. Some workers consider vaccination to be highly efficient but it is generally regarded as unsatisfactory.

Murray et al. (1929) concluded that vaccination against S. cholerae-suis infection is of doubtful value. Even if immunity did develop it was weak and of short duration. Shanks & Lamont (1938) also considered vaccination unsatisfactory. Slavin (1942) carried out a limited vaccination experiment, and although the number of pigs used was too small to be conclusive, the results were not encouraging. Josland (1954) found that vaccination produced a poor and inconsistent somatic antibody response and the results of challenge trials were disappointing. On the other hand Santagostino (1937) found vaccination effective against infection with S. cholerae-suis.

Schofield (1944) considered that inoculation with killed vaccine was effective both for prevention and cure. Genev & Slavkov (1956b) reported that vaccine with adjuvant had good immunogenic qualities. Steffen & Szaflarski (1959) reported the results of vaccination of 7,766 pigs with S. cholerae-suis formolized (0.5% formalin) vaccine. Although vaccination did not protect pigs completely against S. cholerae-suis, it diminished losses. These authors pointed out that since salmonellosis is a disease of young pigs and the immunity following vaccination lasted approximately three months, vaccination should reduce the risk of infection in the most critical period of the pig's life. They considered vaccination to be of special value in fattening centres. Control of salmonellosis by means of hyperimmune serum (used in doses of 50 ml. daily for 2–3 days) was regarded by the same workers as of questionable value, as its protective effect did not last long.

Antipin (1959) reported the successful protection of piglets (27 days old) against experimental infection with S. cholerae-suis by administration of formolized vaccine to pregnant sows (three intramuscular inoculations). Agglutination titres developed in blood and milk of vaccinated sows and in blood of piglets after they received colostrum (the milk titre fell within 20 days after farrowing). The author recommended the vaccination of sows between the 70th and 80th days of pregnancy and of piglets at 20–25 days of age.

2. Treatment

While in some outbreaks the slaughter of severely affected pigs may be the best policy, various sulphonamides and antibiotics have been reported to be of value in treatment.

Slavin (unpublished, 1941) and Kernkamp & Roepke (1942) reported favourable results in treatment of necrotic enteritis with sulphaguanidine. Kernkamp (1945) found this drug of value in doses ranging from 0.75–1.5 g. per 10 lb. body weight and when the treatment was continued over six days. This worker also reported good results with sulfasuxidine (doses 1–1.5 g. per 10 lb. body weight, continued for 6–8 days) and with phthalylsulphathiazole (in doses of 0.65–1 g. per 10 lb. body weight and administered for at least 6–8 days).

Gordon (1951) found two intramuscular injections (doses: 30 ml. per 100 lb. body weight) of 33% sulphamezathine sodium effective in the treatment of S. dublin infection in pigs.

Guthrie (1952) reported favourable results when pigs experimentally infected with S. cholerae-suis, were treated daily for 2–4 days with 0.25–0.3 g. of nitrofurazone. Only one of 19 treated pigs died. Felgate & Swann (1956) also found this drug to be of value in the treatment. Similar findings were reported by Battelli et al. (1959) who used 0.02% nitrofurazone, mixed in the food for five consecutive days. Lannek & Brag (1954) reported that daily doses (0.1–0.5 g.) of this drug, given over 2–3 weeks, would protect pigs in an infected herd. Field (1958) considered that the addition of nitrofurazone as a prophylactic to the diet of pigs in age group: 2–4 months had much to commend it.

Fortushnýi & Gladenko (1957) using streptomycin (200,000 units per kg. body weight) obtained a 68.5% recovery rate in treated pigs.

Hurst & Doyle (1956) reported that "SMP–1" (an experimental drug chemically similar to chloramphenicol) cured experimental salmonellosis in doses of 150 mg./kg./day, for five days and that it prevented infection when given to pigs before exposure. "SMP–1" stopped scours in pigs, while chloramphenicol had little or no effect. Steffen & Szaflarski (1959) reported on the use of chloramphenicol (administered in combination with vaccination against S. cholerae-suis infection) but they obtained good results in only a few of the treated pigs.

Fortushnýi & Gladenko (1957) found that biomycin gave the best results in the treatment of swine paratyphoid. Of 93 pigs treated with this antibiotic (administered orally in doses of 30 mg. per kg. body weight, three times daily for 4–6 days), 91 recovered (97.8%). They also found levomycin to be effective (doses being the same as those of biomycin) and of 102 treated pigs, only four died (96.1% recovered).

Kernkamp & Lindorfer (1959) used a neomycin sulphate preparation in doses 10–12 g. (220-264 mg. equivalent of neomycin sulphate) per pig, for 6-7 days and found a high recovery rate in severely affected pigs.

PREVENTION AND CONTROL

An important feature of paratyphoid in pigs is that recovered animals may become carriers and harbour and spread infection for a long period (Lavers, 1954): it is also accepted that apparently healthy carriers

are present in many herds. Unfortunately there are no reliable methods of diagnosing salmonella infection in live pigs, and the presence of symptomless carriers makes the prevention and control of the disease difficult. No reliable interpretation of the incidence of salmonellosis in pigs can be obtained from serological examination of blood samples, as many healthy pigs carry agglutinins to the common serotypes (Lovell, 1932 & 1934). Rectal swabbing is also unreliable and negative results do not mean that the pig is not infected. In a severe outbreak of S. cholerae-suis infection investigated by one of us (M.G.), rectal swabs from the entire herd did not reveal a single salmonella, yet S. cholerae-suis was isolated in profusion from all internal organs when two of the sick pigs were destroyed about 90 min. after. It is therefore sound policy always to separate newly purchased pigs. The Bulgarian workers, Genev & Slavkov (1956a) reported that an allergic test (using an allergen prepared from lysed S. cholerae-suis cells and applied intradermally to 622 naturally and 23 experimentally infected pigs) appears to be a specific diagnostic aid in detection of salmonellosis. In experimental infection, reaction to this test appeared before the development of clinical signs. However, without personal experience of this test, an opinion cannot be offered as to its value for diagnosis of salmonellosis in live pigs.

Another factor in the epidemiology of this disease is that it attacks young pigs whose resistance has been lowered by a concurrent disease or by poor housing or nutritional deficiency (Field, 1959). It is, therefore, very important to give proper attention to management, housing and the control of any other disease which would lower resistance.

Vaccination as a preventive measure does not appear to be very efficient but the use of a therapeutic agent as prophylactic is regarded as a possible method of control by Field (1958) who suggested the addition of nitrofurazone to the diet of susceptible pigs between two and four months of age.

During an outbreak, isolation and treatment of the sick and in-contact animals, and strict hygienic precautions, are recommended, and, if at all possible, the affected pigs should be provided with a separate attendant. Any obvious faults in management and housing or other adverse factors affecting the general well-being of the pigs should be attended to. Emaciated pigs suffering from chronic disease should be slaughtered, as they will never become profitable and can often act as carriers; they should never be mixed with other pigs, especially young ones.

The contaminated pens should be thoroughly scrubbed, disinfected and left empty for as long as practically possible, especially where there are wooden partitions. The survival time of S. cholerae-suis outside the animal's body is not known, but the massive isolations of salmonella from dry fertilizers and feeding stuffs (Report, 1959b) suggest that this organism, although not a sporulating one, can survive even under adverse conditions.

PUBLIC HEALTH ASPECT

There is ample evidence that salmonella food poisoning in man can

originate in food animals themselves, especially in pigs.

S. cholerae-suis, although the most common serotype in clinical salmonellosis in pigs, is seldom incriminated in food poisoning in man. The report on outbreaks of food poisoning in England and Wales, in 1958, (Report, 1959a) showed that of a total of 4,952 incidents of human salmonella infections, 3,406 were associated with S. typhi-murium and only eleven with S. cholerae-suis. S. cholerae-suis is nevertheless a danger to public health, and outbreaks such as that described by Müller (1931) have been traced to affected animals. S. cholerae-suis appears to be particularly invasive and pathogenic in man. Edwards et al. (1948) pointed out that although this serotype comprised only 5% of isolates from human beings during the period of their observations (1934–47), it was found in 45.6% of blood cultures, and in 41.3% of known fatal cases in man. They also mentioned that among 149 S. cholerae-suis cultures of known origin isolated from man, none were from carriers. Symptomless human carriers of S. cholerae-suis are in fact extremely rare.

Since the first description of the "hog cholera" bacillus by Salmon & Smith (1886) (who named it: "the bacterium of swine-plague"), there have appeared many reports of food poisoning outbreaks as well as sporadic infections in man by S. cholerae-suis (Trawiński, 1948 and 1959, reviewed literature). In 1902, Longcope published a report of two cases of human infection by "paracolon bacillus" which was found later (by other workers) to be S. cholerae-suis. Lütje (1911) described an outbreak of food poisoning due to S. cholerae-suis occurring after the consumption of pork derived from a sick animal. Harvey (1937) reviewed the literature on S. cholerae-suis infection in man and reported 21 new cases. Boycott & McNee (1936) described a fatal case and Schwabacher et al. (1943) reported two cases of generalized infection with this organism. Recently Baczyński & Szaflarski (1959) gave an account of an outbreak of food poisoning due to S. cholerae-suis, involving 19 people after eating infected pork, which was suspected of being insufficiently sterilized.

In addition to S. cholerae-suis and S. typhi-murium (both encountered in clinical disease in pigs, Buxton, 1957) pigs, as already mentioned, are known to be an important reservoir of a variety of other serotypes.

From the public health point of view, it is highly significant that salmonella are isolated frequently from mesenteric lymph nodes of apparently healthy, slaughtered pigs and that many serotypes recovered from pigs, appear also on the list of isolates from food poisoning outbreaks in man. Furthermore mesenteric lymph nodes of pigs are more commonly infected than those of any other species of food animal (Smith, 1959).

Pork and its products are more commonly associated with outbreaks of food poisoning than the meat of other animals (Report, 1958). Processed and "made-up" meats were vehicles of infection in about half of the outbreaks of salmonellosis in man traced to a specific item of food (Report, 1958). Investigations during the four years referred to in this report, showed that nearly two-thirds of incriminated processed and re-heated meat dishes were pork pies, brown sausages and cold pork. Many other reports of the transmission of salmonellosis by pork products are to be found in literature (see Trawiński, 1948). One of many is that

of Hauser et al. (1945) on an outbreak of food poisoning by S. berta, after consumption of contaminated smoked sausage and also an outbreak of salmonellosis involving over 450 people in Co. Durham which was traced to sandwiches, sausages and pies made from a pig's carcass infected with S. newport (Report, 1950).

Another outbreak of food poisoning involving 63 patients in a hospital was described by Primavesi (1956). It occurred after the eating of sausages prepared from pork from pigs infected with S. heidelberg.

Similar outbreaks after consumption of brawn (sausage) infected with S. anatum and affecting 320 people were described by Takács & Kneffel (1955).

The incidence of salmonella in carcasses of pigs passed as fit for human consumption in the U.S.A. is high. Felsenfeld et al. (1950) examined 573 samples of pork from shops which had been inspected and passed for human consumption and found that 14.3% (see original article) yielded salmonella. From 101 uninspected pork samples they isolated salmonella from 27. Of 102 samples of hamburger, 18 were infected but only one of 512 samples of beef yielded salmonella. Cherry et al. (1943) investigating the incidence of salmonella in retail meat products, found the incidence to be higher in pork than in beef. Of 170 pork products examined, 5.9% were infected. The largest yield of salmonella was from liver (20%), followed by brain (10%) and then pork chops (9.5%). Only one instance was found of fresh sausages yielding salmonella. Galton et al. (1954b) isolated salmonella from 51 of 217 samples of fresh pork sausages. Of 127 samples of smoked sausage, 16 (12.5%) were positive for salmonella. These workers isolated a total of 19 serotypes. S. derby and S. anatum were the commonest, and S. cholerae-suis was isolated eight times. In Germany (district of Thuringia) Goerttler & Richter (1957) examined bacteriologically 140,000 samples of meat from 1945 to 1955 and isolated 735 strains of salmonella; 275 (35.5%) were from calves, the commonest source, 178 (26.5%) from pigs and 167 (23.5%) from cattle. The strains that were isolated most frequently from pigs were S. typhimurium (42.1%) and S. enteritidis (26.8%).

Jørg (1957), in Switzerland, examining 8,000 pigs from 198 herds which were slaughtered to control swine fever, isolated S. cholerae-suis from 320 animals (4%) in 46 herds: 75% of the isolates were from organs (when the carcass was passed as fit) and 25% from muscles (in these cases the carcass was condemned).

In Poland, Kafel (1957), by direct plating of samples onto solid media, isolated salmonella from 4.1% of 5,182 pigs slaughtered during 1953–54. A similar examination of 6,308 pigs in 1955 yielded 545 salmonella strains (8.64%). Kafel did not examine the liver in these investigations. But in the first eight months of 1956, he included in his examination sections of liver, spleen, kidney and lymph nodes and portions of muscle from the fore and hind limbs. He incubated preliminary cultures in bile-broth for 18 hours before plating on MacConkey and Conradi-Drigalski solid media. Of 4,917 pigs 696 (14.5%) yielded salmonella, 203 (29.16%) in the portal system. Kafel showed in a series of examinations that salmonella in the internal organs did not invade the skeletal muscles for 48 hours after slaughter when the carcass

was kept at 4°C., but could do so from the lymph nodes. When salmonella was isolated originally in the skeletal muscles and carcass, it could not be found after storage for 48 hours at 4°C.

Newell et al. (1959), in Northern Ireland, found salmonella in 3% of pork samples and 70% of samples of pork sausage.

During a survey in six abattoirs in England from 1950 to 1953 (Report, 1955), S. dublin was isolated at one laboratory (Newcastle) from six of 452 samples of bile and from the liver and a lymph node of one of 27 pigs. This serotype was not isolated by the other laboratories, which reported negative results in 500 samples of bile, 1,130 samples of liver and 687 samples of spleen. Swabs taken from the peritoneal surface of 1,146 sides of pig carcass yielded three strains of salmonella, two S. typhi-murium and one S. anatum.

McDonagh & Smith (1958) examined bacteriologically the untreated liver, spleen and mesenteric lymph nodes of pigs slaughtered at Bradford abattoir during 1954–56, and found salmonella contamination in nearly a quarter of the tissues. The combined figures for several series of examinations showed that while the rate of surface contamination was 18%, the true rate of tissue infection was 4%. Six different serotypes were isolated, the commonest being S. typhi-murium and S. derby.

Another important aspect, stressed by Gaugusch (1957), is that inspectors pass meat as fit for human consumption on appearance and odour and examine only a few samples bacteriologically. Most samples of pork and its products responsible for food poisoning showed no apparent changes, and aroused suspicions neither in the meat inspector nor in the consumer.

Müller (1957) considered that the recent increase in infections with uncommon salmonella types in Denmark, especially in calves and poultry, was probably due to the import of large quantities of animal feeding stuffs in that country after the second world war. Lerche (1957) pointed out that meat-meal and fish-meal contaminated with salmonella have infected pigs and poultry and that they are responsible for many latent infections.

Schaal (1957) found eleven rare salmonella types in 5% of faecal samples from healthy slaughtered pigs, and concluded that the infections originated in imported feeding-stuffs. Adam (1957) isolated salmonella from 15.1% of 924 samples of fish, meat and bone-meal. Most of the infected samples were imported.

In England, Walker (1957), examining 123 samples of organic fertilizers, found salmonella in 40%. There were 34 serotypes, 28 of them from bone-meal. He pointed out that such products may be a source of unexplained outbreaks of salmonella infection in man and animals. A recent survey (Report, 1959b) of 1,262 samples of animal feeding-stuffs and organic fertilizers, both imported and produced in the United Kingdom, revealed salmonella in a wide variety of products including raw and processed material. A high incidence was found in imported bones and bone products and in Angola fish-meal. Altogether 88 salmonella serotypes were isolated, including six new types.

In Northern Ireland, Newell et al. (1959) found salmonella in 24% of samples of pig meal collected from five farms. The same types were found in the meal at the mill, and in fish-meal and bone-meal before

mixing. The authors suggested that some salmonella types pass from feeding-stuffs through pigs to human food and cause human infection.

Feeding-stuffs with such a high rate of salmonella contamination probably cause infections in pigs. The infections may be latent, since there is no evidence of any recent increase in outbreaks of clinical salmonellosis due to types other than S. cholerae-suis and S. typhimurium. No doubt there is a link between these feeding-stuffs and the high infection rate of the mesenteric lymph nodes of pigs. Further research work should establish such a link and show that it is related to some of the outbreaks of food poisoning in man.

Infection of pork and its products is due primarily to infected pigs, but meat from uninfected carcasses may become contaminated at various stages of preparation. It may be splashed with faeces, or bile from a punctured gall bladder, or it may be handled with contaminated equipment. After it leaves the abattoir it may be infected by rats and mice, or by human carriers of salmonella infection.

Methods of cooking pork and its products, especially sausages and pies, may be inadequate. The temperature and the period of cooking may be insufficient to destroy salmonella in the depth of the meat, particularly in a large portion.

The number of salmonella organisms present at the time of consumption is important (Howie, 1957). The work of McCullough & Eisele (1951 a, b, c), experimenting with human volunteers, showed that a large number is required to set up infection and that the type of salmonella is significant.

Howie stated: "the mere presence of food-poisoning bacteria in the food is not the whole matter: often it is their opportunity to grow freely within the food that causes the trouble". Infected, but freshly prepared food, may contain too few salmonella to set up infection but if it is left in a warm room, the organisms will multiply and in a short time make the food dangerous. The importance of storing food at low temperature is apparent.

It appears that the control of salmonellosis calls for the fullest co-operation between workers at the various stages: on the farm, in the abattoir, in the food factory, the canteen, the shop and the home. These include farmers, veterinary surgeons, doctors, diagnostic laboratories, public health authorities, meat inspectors, and slaughterhouse and canteen personnel. Each sees a different aspect of the problem and the whole story can be told only by collecting the experiences of workers in the control and prevention of animal diseases, the production and sale of human food and the protection of human health.

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Veterinary Reviews and Annotations
VOL. 7, PT. 1 (April 1961)

The Bovine Uterus—Histopathology

BY

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General tissue changes. A good account of the earlier work on uterine histopathology was given by Frei (1925). He recognized a large number of types, of which only the acute and chronic purulent forms, including pyometra, the cystic and the necrotic types have been distinguished by later workers. Frei reported that the degree of catarrhal-purulent endometritis varied in different parts of the uterus. He described, in the early stages of the chronic purulent form, foci of polymorphs below the surface epithelium and collecting round the glands. Lymphocytes might be frequent between the glands. As the condition progressed the glands sometimes filled with polymorphs, and in some cases disappeared. In pyometra, the endometrium was also visibly ulcerated, and lacked surface epithelium, but occasionally several layers formed in regenerating areas. In some uteruses the endometrium contained pea-like abscesses.

Frei considered necrotic endometritis to be characteristic of the acute septic puerperal disease; a diphtheritic membrane of necrotic tissue might form, become gangrenous and desquamate, and portions of it would block the cervical canal. He did not link cystic endometritis with cystic ovarian disease. The characteristic change was the clustering of leucocytes (probably mostly round cells) both in and around the gland cavities, with the disappearance of the gland epithelium and general fibrosis in the stroma. The cystic appearance of the glands was attributed to blockage of the ducts with shed epithelium.

These observations have been confirmed and supplemented by a number of workers. László (1935) described permanent lymph follicles in the chronic purulent form, with fibrotic replacement of superficial glands and their infiltration with polymorphs. Erismann & Saxer (1933) confirmed László's finding that the deeper glands (? ends of coiled glands) may remain intact. They also observed massive infiltration of polymorphs. Cembrowicz (1946), however, found, as Frei did, that both the general and the glandular infiltration was of a mixed type, and that many round cells participated. Lymph nodules were observed by Simon & McNutt (1957b) and by Moss et al. (1956). Neither of these teams reported polymorphs, but Schnider (1958), like Cembrowicz, recorded a mixed infiltration of lymphocytes and polymorphs. An increased number of plasma cells was observed by Simon & McNutt (1957b), by Baur (1951) and by Schnider; and de Bois & van den Akker (1957) found that their proportion rose with age. In pyometra, plasma cells were described by Tagliavini (1935); he claimed in opposition to Frei that the surface epithelium often survived, in a vacuolated form. In other respects he confirmed the findings of Frei, as did Cembrowicz in seven cases, and

Quinlan (1929) in four.

Cystic endometritis was encountered by Tagliavini. In some cases it was associated with ovarian cysts, which he dismissed as coincidental. He considered hyper-secretion to be the cause, as well as duct block by plasma cells. Erismann, Saxer and Quinlan followed Frei in attributing uterine cysts to duct block, although some of Quinlan's animals had ovarian cystic disease. Laszló too noted ovarian cysts in some of his cases of cystic endometritis. Like Frei, László stressed the virtual absence of polymorphs, the infiltration consisting mainly of round and plasma cells; Quinlan agreed, and also noticed some eosinophiles and macrophages. However, Queisser (1941) examining 14 cows with cystic ovarian disease, found cystic endometritis in 13, characterized by a rich polymorph infiltration as well as by glandular dilatation and stromal oedema. This contrast suggests that two types of cystic endometritis may exist, of which at least one is certainly associated with cystic ovarian disease; Dawson (1958) observed cystic glandular dilatation in over 40% of 48 cows with cystic ovaries: probably a minimum figure, since most of the remaining 60% showed mixed infiltration and fibrosis severe enough to mask any cystic glandular dilatation that might have been present. A few cases of cystic dilatation were observed by Simon and McNutt in 109 infertile cows and by Moss et al. in 40 infertile cows.

Tissue changes studied by biopsy. During the period under review, more effort has been directed to the evolution of biopsy techniques than to their application for research purposes. Three types of instrument appear to have been used, the first being that attributed to Folmer Nielsen around 1930. This consisted of a tube, containing an extrusible plunger having a trough near its tip, which consisted of a cutting edge facing towards the rear. In use the uterine wall is pressed into the trough by the fingers per rectum and a sample detached into the trough by retraction of the plunger within the tube. Brus (1952), Strikwerda (1956) and Schnider all used instruments on this principle, Brus, comparing his samples with post-mortem specimens, claimed that a representative sample was obtained, but Schnider attached importance to the differences which both observed, the chief of which was the appearance of masses of detritus in the gland lumina of biopsy specimens. Miller's (1951) instrument relied on suction from a 40 ml. syringe to draw the sample into the trough; slight finger pressure was used as well. Such an instrument was used very successfully by de Bois & van den Akker (1957). Good biopsies were obtained but the full depth of the glands was not reached. Grunert (1956) used a modification of this instrument which cut deeper, but sometimes resulted in excessive bleeding. Rommel (1959), modifying this principle further, produced a slimmer instrument which was suitable for dioestrous heifers and could be used by a single operator. Zurgilgen (1948) resorted to a completely different principle. In his instrument the forceps-like action of two sharp scoops was controlled remotely through the long tube. It was inclined to penetrate deep into the muscle layers, but the principle was used successfully by Széky & Dózsa (1955, 1956) and by Skjerven (1956), who found that an instrument of Brus' type bruised the glands and caused the

lining and surface epithelium to slip. Skjerven's modification of the forceps principle was designed to penetrate less deeply, and included a net to catch the sample. This instrument is probably the best, but it is complicated. The simpler instruments appear to detect the grosser forms of tissue damage fairly accurately.

Széky and Dózsa observed most of the changes characteristic of chronic purulent endometritis in biopsy samples. They described the destruction of the glands after these had filled with inflammatory cell elements, which remained to mark the sites.

Incidence and significance of tissue changes. In assessing incidence, account must be taken of the probability that many cases resolve by the time the animals are culled. Information on the extent to which cases resolve might be obtained from the incidence in cattle with a history of infertility. Random abattoir samples represent only the most severe cases.

Incidence assessed from post-mortem specimens. There are no complete histological data on the uterus of random slaughter cows. The usual practice has been to select for histological study uteruses that were abnormal macroscopically. In this way László studied histologically 34 abnormal uteruses from 300 random slaughter cattle (11%), Aziz-ud-Din (1948) reported 35% in a series of about 230, while Cembrowicz noted less than 6% in 1,030. Bhattacharya et al. (1954) found abnormalities in 27% of uteruses from 1,020 buffaloes. In these studies, practically all uteruses abnormal to the naked eye showed gross damage under the microscope. On the other hand, evidence exists that many uteruses with important microscopic lesions may be unchanged macroscopically (Dawson, 1949). This range of 6–35% represents a minimum incidence of such cases in random samples, and denotes the economic importance of the condition.

In a histological study of material from 252 cows discarded for failure to breed, Dawson (1958) described oviduct lesions in 52% and produced evidence that the chief cause was the ascent of uterine infection. Yet in 32% of these cows with salpingitis the endometrium was histologically normal at slaughter, whereas at least as great a percentage of infertile cows with normal oviducts had lesions of endometritis. This emphasizes the recuperative ability of the endometrium during the final lactation period. Erismann and Saxer, using Frei's criteria, claimed to observe changes of endometritis in 65 out of 68 discarded cows, and not in normal controls. Roark (1953) noted severe erosion of endometrial tissues in 25% of 52 discarded cows and obstructions of the genital tract in a further 29%: these changes were not apparent in 33 control animals with a normal history. Fujimoto (1956) found simple endometritis in 45% of 87 discarded cows and endometrial glandular hyperplasia in a further 7%, whereas only 22% of 286 cows taken at random had comparable lesions.

The majority of cows culled through infertility have permanent lesions in either the endometrium or the oviducts, while in others an endometritis has probably healed by the time of slaughter. It seems probable, however, that many cows are rendered temporarily infertile by

an endometritis which later resolves partially or completely. Some light was shed on two aspects of this question by the work of Moss et al. (1956), who studied uteruses from 25 normal cows and 30 with a history of infertility. With regard to the first aspect—the correlation of lesions with breeding history—only four of the 25 normal cows had typical tissue changes and none had lymph nodules in the endometrium, whereas 21 of the 30 infertile animals had abnormal tissue and eight had lymph nodules as well. With regard to the second aspect—the amount of damage allowing normal reproductive function—seven of eight uteruses with lymph nodules contained normal embryos, conception having followed a period of infertility. Statistically, the most significant lesion of infertility was encapsulation of endometrial glands. A second series of 12 normal and ten infertile animals from another locality yielded typical tissue lesions in only two of each group, but the numbers were small. However, Simon & McNutt (1957b), examining 109 cows that had been served in vain four times, found pronounced endometritis in only one and minor abnormalities in 49. This low incidence of lesions is surprising in a large series of animals.

Significance assessed by biopsy. Biopsy is the method of choice for investigating both the correlation between infertility and lesions, and the degree of damage compatible with fertility. But first it must be established whether a single biopsy sample can be taken as representative of the endometrium as a whole. Széky and Dózsa mentioned that this is accepted in human practice. Brus, comparing biopsy with post-mortem samples, one from the body of the uterus and one from each horn, claimed that biopsy samples were fully representative; van der Kaay (1950) accepted this too. Moss et al., however, noted in their material a higher incidence of abnormality in the body than in the uterine horns. Deininger (1957), comparing post-mortem samples from these two sites with single biopsy samples in 36 cows, concluded that biopsy samples from the base of the uterine horns were at best 80% representative; the main source of error was patchy distribution of cystic glandular dilatation. Van den Hoek (1959) studied the question systematically in ten cattle checked carefully for normality. Serial sections of the entire horn area were examined and accurate counts made of leucocytes, other phagocytes, mast cells, and plasma cells. Significant differences were found between horns (even in the same cow) which had or had not been pregnant, but despite other minor variations in cell distribution his conclusion was that a single biopsy gave a reasonably accurate index, though the count error might reach 40% for plasma cells, considered important in disease as the actual producers of local antibody. A general conclusion from all this work might be that the index is slightly less accurate in abnormal material and that a biopsy from each horn is much preferable to single sampling.

Brus took biopsy samples from 100 cows when they had returned twice to service; he analysed the tissue changes and correlated them with the subsequent service history. He established a simple grading for the inflammatory changes shown by the biopsies; type A included only a few inflammatory cells near the surface; in type B there were many cells

uniformly distributed, while in type C the very numerous cells formed foci round glands or vessels (the "significant lesion" according to Moss, Sykes & Wrenn). Overall only 29 of the cows showed no inflammatory changes. Two subgroups were distinguished, one including 41 cows either from Brucella-infected herds or with trauma at the previous parturition, the second containing 59 with normal histories up to the current service period. The samples from the two groups were very different. In the group with Brucella infection or trauma 20% of the biopsy samples were normal, 50% were of types A and B and 30% type C. In the normal group 36% of the samples were normal, 53% of type A, and only 9% of type B and 2% type C. This and the results of subsequent service suggested that persisting endometritis was seldom here the cause of infertility. In the cases of both groups that were followed up, type A changes interfered little with reproductive function: the 100 cows conceived and carried within six weeks of biopsy, of these 20 had yielded normal biopsies, 33 those of type A and only six those of types B and C. Nine cows conceived three months or more after biopsy, samples from four of which had been classified as B or C, while of 19 discarded as permanently infertile, two had shown normal biopsies and ten those of types B and C. Brus thus demonstrated that endometritis, as represented by B and C samples, was frequently associated with trauma, and that recovery was poor. Only ten of 30 cases were known to breed again, and ten were culled as barren.

De Bois and van den Akker took biopsy samples from both horns of 137 cows about 13.5 weeks post-partum shortly before service. They diagnosed endometritis only when samples contained foci of inflammatory cells, thus excluding those corresponding to Brus' A and B grades. The positive samples were graded according to the number of foci per section, 1–2 foci representing grade I, 2–5 grade II, and over 5 grade III. Of the 57 cows holding to first service, 28 yielded normal biopsies, 18 those of grade I, 9 of grade II and 2 of grade III, suggesting that a moderate degree of tissue change need not always delay conception. The following indicates the prognosis of the histological findings.

Prognosis correlated with diagnosis of endometritis by biopsy from de Bois & van den Akker (1957).

Type of Biopsy	Number	% holding to first insemination
Negative	61	45.9
I	39	46.1
II	21	42.8
III	16	12.5
Total	137	

These results imply that some signs of endometritis occur in actually a small majority of cows at 13.5 weeks post-partum, and that a significant depression of the conception rate is reached only at grade III. Fertility was much higher in relation to the severity of the lesions than in Brus' material. This might be partly due to the shorter interval between calving and the time of biopsy. Evidence in favour of this view was provided by the results of repeat inseminations, by which time the differences in conception rate were largely eliminated. The material of de Bois and van den Akker, like that of Brus, included a sub-group with trauma at the previous parturition. Of 31 cows 29 had histopathological signs, and 21 bred again, requiring a mean of 21 weeks between date of last calving and conception, compared with the general mean of 17 weeks.

These two investigations suggest that the prognostic value of biopsy is much greater by the time infertility is recognized, presumably because the tissue lesions are then of a more refractory nature than those detected before service. Brus considered the prognosis worse when lymphocytes predominated over polymorphs in the infiltration, but at the earlier stage de Bois and van den Akker noted mainly lymphocytes and some eosinophiles, which did not seem to affect the conception rate in many cases.

The effects of specific infectious agents. The histology of tuberculous endometritis has been investigated more fully than that of any other infection. Quinlan (1929) observed two distinct forms, true tubercles lying deep in the uterine wall, and pinhead size miliary lesions apparently on the surface of the mucous membrane. Klimmer, Haupt & Roots (1929) found 9 of 12 tuberculous uteruses to be affected with the miliary type. Nielsen (1934) described four degrees of advancement of the lesions in the uterine wall, together with their clinical indications. Stamp (1944), who made an intensive study of this infection throughout the body, explained that the miliary involvement resulted from infection descending directly from the peritoneum through the oviducts, while the deeper true tubercles were due to blood-borne infection. Cembrowicz found both types of lesion, and observed that the miliary lesions often involved the glands. He thought that direct infection of the oviducts was possible from tubercles originating in the deeper layers. Baur (1951) observed only the miliary type of lesion, in 6 of 30 infertile cows. Typically, the foci were rich in lymphocytes, giant and epithelioid cells. Schnider refers specifically to the diagnosis of uterine tuberculosis by biopsy. He describes the samples in his cases as "highly characteristic", but gives no further details beyond mentioning that fibrosis was conspicuous, whereas it was not in other types of endometritis. Presumably, epithelioid and giant cells were conspicuous also.

There are two detailed studies on the changes in the uterus in vibriosis. Simon & McNutt (1957a) found endosalpingitis, but no pronounced endometrial changes in three heifers killed up to 11 days after the injection of Vibrio fetus into the uterus during oestrus. Heifers infected in about the fifth month of pregnancy showed a variety of changes; erosion of epithelium, necrosis, oedema, haemorrhage and infiltration with polymorphs and lymphocytes, with the appearance of many plasma cells.

Huysman (1957) reported a normal picture within three weeks of an infective service, but changes between the third and sixth weeks: filling of the gland lumina with necrotic nuclei, and infiltration of lymphocytes and a few eosinophiles. These changes, which regressed from the ninth week, were not observed in animals treated with streptomycin.

Dawson (1949) described the changes in 19 cows which had yielded uterine pus with a high concentration of Corynebacterium pyogenes. At post-mortem examination five yielded pus, two of them from large endometrial abscesses; a further 12 exhibited ascent of infection to the oviducts. All the uteruses showed considerable microscopic changes. A typical finding was the replacement of large areas of endometrium by dense fibrosis. Sections rarely showed superficial glands. A few surviving deep glands, surrounded by fibrous rings, had necrotic epithelium and contained many polymorphs and lymphocytes. Cells of this type were common also in surviving stroma. Baur confirmed these results, reporting in four animals with C. pyogenes endometritis "accumulations of plasma cells, fibroblasts and polymorphs, and necrotic foci". He did not emphasize the occurrence of lymphocytes. Baur claimed, perhaps on insufficient evidence, that his histopathological findings in the uterus of each of his groups of cattle with tuberculosis, C. pyogenes, Trichomonas foetus and Streptococcus pyogenes infections were typical. He believed that mixed infiltration of lymphocytes and polymorphs was rare, and that lymphocytes and eosinophiles characterized a non-purulent hyperaemic reaction; this coincides with Huysman's description of the changes in vibriosis as well as those Baur found in 12 cases of trichomoniasis. Nevertheless, pyometra is a typical lesion in trichomoniasis. De Bois and van den Akker attempted to correlate bacterial with pathological findings in all their material. This was possible only in cases of severe disturbance of function leading to permanent infertility, with the early appearance of clinical symptoms. Beta-haemolytic streptococci were recovered predominantly, and were associated with a high density of the inflammatory foci which these workers used as their criterion.

Descriptions of the changes in the empty uterus during or after brucellosis are rare. Loppnow (1960) in an article on the pathology, anatomy and histology of Br. abortus infection did not refer to the female genitalia. Wagener & Bisping (1959) described the changes very briefly as "oedema, infiltration and necrosis". Payne (1959) described the changes in the pregnant uterus as essentially destruction of the glands, invasion by large numbers of lymphocytes, plasma cells and polymorphs and the appearance of oedema fluid in the stroma. Payne (1960) found no brucella in the empty uterus of heifers infected intra-ocularly, though Munro has indicated that intra-uterine infection may cause infertility rather than abortion.

Effects of disturbances on endocrine function. Széky & Dózsa, also Ajello described histologically the uterus of animals with true retention of the corpus luteum. They agreed that the glands showed proliferation. Ajello found increased vascularity while the Hungarian workers observed numerous deposits of haemosiderin

in the stroma. They reported round cells and polymorphs in the stroma of some of their cases, whereas only two of Ajello's 16 had clinical purulent endometritis and only in these were secondary inflammatory changes evident. Ajello considered a persisting corpus luteum to be usually actively functional. But persistence is often associated with pyometra (especially in the earlier literature) as are also small empty ovaries and ovaries with cystic disease. Ajello's view therefore requires modification.

Conclusions. Economic benefits of the study of histopathology of the uterus are the diagnosis and prognosis of endometritis. Biopsy is the chief method of study and further work on the lines pioneered by Brus and by de Bois and van den Akker is essential. It would be valuable to repeat de Bois and van den Akker's work on cattle of normal fertility, but prevented from mating for the period allowed by Brus before sampling. This would determine the importance of the time of sampling, in producing differences between the results of Brus and those of de Bois and van den Akker. Useful information on prognosis might be obtained by taking samples from 300 normal cattle 13.5 weeks after calving and additional samples from all of them not conceiving to the first three inseminations. The additional samples would yield material similar to that studied by Brus, and might indicate the importance of factors other than endometritis in causing infertility. More post-mortem work is required, not only on animals pregnant after a period of infertility such as those studied by Moss et al., but also on random abattoir samples, since the incidence of microscopic lesions is not well established. The type of cell infiltration associated with cystic endometritis has not been investigated fully, and this may be important in view of Brus' suggestion that the presence of lymphocytes may worsen the prognosis.

Continued study of the effects of infectious agents might improve the possibilities of diagnosis by biopsy; the changes have been described fully only in Vibrio fetus infection and in tuberculosis. Terpstra (1956) produced infertility in heifers by inoculating them with Strain 19 Br. abortus vaccine intracervically, but Payne using the ocular route was unsuccessful. Biopsy and post-mortem study of cattle inoculated by Terpstra's method should prove interesting. A rapid field method for preparing biopsy samples would be very helpful.

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Part 2 of Volume 7, which will be published in October 1961, will therefore be the last issue of *Veterinary Reviews and Annotations.*

As from January 1962, review articles of the same type as those at present published in *Veterinary Reviews and Annotations* will be included in the *Veterinary Bulletin.* The amalgamation of the two journals will not lead to any reduction in the number of abstracts, nor is it the intention to reduce the number of reviews. The new arrangement giving both abstracts and reviews under one cover will, we hope, be more convenient to our readers.

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